

WHAT IS CLAIMED IS:

1. An amplification-based method for producing a promoter-containing siRNA expression cassette, comprising:
  - i) treating one strand of a double-stranded promoter sequence, in an amplification reaction mixture, with an oligonucleotide primer which is complementary to the 5' end of the promoter sequence;
  - ii) treating the other strand of the promoter sequence, in the amplification reaction mixture, with a second oligonucleotide primer which is complementary to the 3' end of the promoter sequence, wherein the second primer comprises one or more sequences which are complementary to a sequence encoding a sense and/or antisense sequence of a siRNA molecule, along with one or both of a loop sequence and a terminator sequence; and
  - iii) treating the amplification reaction mixture of steps (i) and (ii) in an amplification reaction at a temperature for annealing and extending said primers on the promoter sequence and at a temperature for denaturing the extension products to provide an amplified product comprising the promoter, one or more sequences encoding the sense and/or antisense sequence of the siRNA molecule, and one or both of the loop sequence and the terminator sequence, and wherein steps (i)-(iii) are repeated a sufficient number of times to amplify the promoter-containing siRNA expression cassette.
2. The method of claim 1, wherein the method is a PCR-based method.
3. The method of claim 1, wherein the promoter is a Pol III promoter.

4. The method of claim 3, wherein the Pol III promoter is a mammalian U6 promoter.
5. The method of claim 4, wherein the U6 promoter is a human U6 promoter.
6. The method of claim 1, wherein the sequence encoding the terminator sequence comprises a sequence of about 4-6 deoxyadenosines.
7. The method of claim 6, wherein the sequence encoding the terminator sequence comprises a sequence of 6 deoxyadenosines.
8. The method of claim 1, wherein the second primer further comprises a tag sequence to identify functional siRNA encoding sequences.
9. The method of claim 8, wherein the tag sequence further comprises a restriction site useful for cloning.
10. The method of claim 1, wherein the second primer comprises a sequence that is complementary to a sequence encoding a sense sequence and a sequence that is complementary to a sequence encoding an antisense sequence of said siRNA molecule, along with a terminator sequence.
11. The method of claim 12, wherein the sequences complementary to a sequence encoding the sense and antisense sequences are attached by a loop sequence.
12. The method of claim 13, wherein the loop sequence contains about 6 to about 9 nucleotides.

13. The method of claim 1, wherein the amplified product comprises the promoter sequence, a sequence encoding either the sense or antisense sequence of the siRNA molecule, and the loop sequence or the terminator sequence.

14. The method of claim 13, wherein the amplified product comprises the promoter sequence, a sequence encoding either the sense or antisense sequence of the siRNA molecule, and the terminator sequence.

15. The method of claim 13, wherein the amplified product comprises the promoter sequence, a sequence encoding either the sense or antisense sequence of the siRNA molecule, and the loop sequence, said method further comprising the step of treating the amplified product, in another amplification reaction, with a third oligonucleotide primer, a portion of which is complementary to the loop sequence of the first amplified product, and which comprises a sequence complementary to a sequence encoding the antisense sequence when the first amplified product contains the sense encoding sequence, or a sequence complementary to a sequence encoding the sense sequence when the first amplified product contains the antisense encoding sequence, along with a terminator sequence, to provide a second amplified product.

16. The method of claim 15, wherein the second amplified product comprises the promoter sequence, a sequence encoding the sense sequence and a sequence encoding the antisense sequence of the siRNA molecule, wherein the sense and antisense sequences are attached by a loop sequence, and the terminator sequence.

17. The method of claim 1, further comprising the step of transfecting a cell with the amplified promoter-containing siRNA expression cassette, wherein an siRNA molecule is expressed.

18. The method of claim 17, wherein the selected cells are mammalian cells.

19. The method of claim 17, wherein one or more of the oligonucleotide primers are modified.
20. The method of claim 19, wherein one or more of the oligonucleotide primers are modified by phosphorylation.
21. The method of claim 17, further comprising the step of screening for a target site on mRNA sensitive to the expressed siRNA molecule.
22. The method of claim 17, wherein the cell is transfected with two or more different siRNA expression cassettes.
23. The method of claim 22, wherein the different siRNA expression cassettes contain one or both of a different siRNA encoding gene and a different promoter.
24. A PCR-based approach in the form of a kit for producing a promoter-containing siRNA expression cassette, comprising a double-stranded, promoter-containing template, an oligonucleotide primer complementary to the 5' end of the promoter-containing template, and an oligonucleotide primer complementary to the 3' end of the promoter-containing template, wherein the 3' primer comprises one or more sequences complementary to a sequence encoding a sense and/or antisense sequence of a siRNA or siRNA molecule.
25. The PCR-based approach of claim 24, wherein the promoter is a Pol III promoter.
26. The PCR-based approach of claim 25, wherein the Pol III promoter is mammalian U6 promoter.
27. The PCR-based method of claim 26, wherein the U6 promoter is a human U6 promoter.

28. A method for screening potential target sequences susceptible to siRNA mediated degradation, comprising transfecting a cell with an amplified siRNA expression cassette under conditions in which an siRNA molecule can be expressed and mediate degradation of the potential target sequences.

29. A method for inhibiting expression of a target gene, comprising transfecting a cell with an amplified siRNA expression cassette under conditions in which an siRNA molecule can be expressed and inhibit expression of the target gene.